

CLAIMS

1. An array of protein-binding agents stably attached to the surface of a solid support, said array comprising:

a solid substrate having a substantially planar surface;

5 a plurality of different protein-binding agents bound to said substrate, each of said protein-binding agents comprising,

an anchoring segment stably bound to the substrate surface,

a peptidomimetic protein-binding segment, and

10 a linker segment connecting and separating the anchoring and peptidomimetic segments.

2. The array of claim 1, wherein said substrate comprises a metal on said planar surface beneath said anchoring segment.

3. The array of claim 2, wherein said substrate is one of glass, plastic or metal.

15 4. The array of claim 2, wherein said metal is one of aluminum, gold or titanium.

5. The array of claim 2, wherein said peptidomimetic segment is a peptoid.

6. The array of claim 2, wherein said linker segment is selected from the group consisting of C2 – C100 aliphatic chains, polyethylene oxide, and orthogonal peptidomimetic or peptide oligomers.

20 7. The array of claim 2, wherein said anchoring segment is a thiol.

8. The array of claim 2, wherein said anchoring segment is biotin.

9. The array of claim 2, wherein said metal substrate surface is further coated with a functionalized one of an amino-modified thiol and a siloxane beneath said anchoring segment.

10. The array of claim 9, wherein said aminothiols or aminosilane is functionalized with a maleimide.

11. The array of claim 10, wherein said anchoring segment is a thiol.

12. The array of claim 9, wherein said aminothiols or aminosilane is functionalized with one of a hydrazide, aminooxy, N-hydroxysuccinimide, anhydride, aldehyde, disulfide, thiol, azide and phosphine.

13. The array of claim 9, wherein said metal substrate surface is further coated with an avidin protein beneath said anchoring segment.

14. The array of claim 13, wherein said avidin protein is selected from the group consisting of avidin, streptavidin, neutravidin and analogs.

15. The array of claim 13, wherein said anchoring group is biotin.

16. The array of claim 13, wherein said avidin protein is attached to the metal substrate surface via an NHS-LC-LC-biotin moiety.

17. An array of protein-binding agents stably associated with the surface of a solid support, said array comprising:

a solid support having a substantially planar aluminum surface coated with a maleimide-functionalized aminothiols or aminosilane;

a plurality of different protein-binding agents bound to said substrate, each of said protein-binding agents comprising,

a thiol substrate anchoring segment stably bound to the maleimide-presenting substrate surface,

a peptoid protein-binding segment, and

an aliphatic linker segment connecting and separating the anchoring and peptidomimetic segments.

18. The array of claim 17, wherein said maleimide-functionalized aminothiols or aminosilanes comprises a spacer.

5 19. An array of protein-binding agents stably associated with the surface of a solid support, said array comprising:

a solid support having a substantially planar aluminum surface coated with an avidin-functionalized aminosilane or aminothiols;

10 a plurality of different protein-binding agents bound to said substrate, each of said protein-binding agents comprising,

a biotin substrate anchoring segment stably bound to the avidin-presenting substrate surface,

a peptoid protein-binding segment, and

15 an orthogonal peptide linker segment connecting and separating the anchoring and peptidomimetic segments.

20. The array of claim 17, wherein said avidin-functionalized aminosilane or aminothiols comprises an NHS-LC-LC-biotin moiety.

21. A method of making an array comprising a plurality of different protein-binding agents stably associated with the surface of a solid support, said method
20 comprising:

preparing for bonding a solid substrate having a substantially planar surface;

contacting a plurality of different protein-binding agents with said substrate under conditions sufficient for said protein-binding agents to become bound to said substrate surface, each of said protein-binding agents comprising,

25 a substrate anchoring segment,

a peptidomimetic protein-binding segment, and

a linker segment connecting and separating the anchoring and peptidomimetic segments;

whereby said array is produced.

5 22. The method of claim 21, wherein said contacting comprises spotting a droplet of a solution of each of said protein-binding agents in a different location on said substrate surface under conditions such that binding of the protein-binding agents to the substrate surface is complete before the droplet evaporates.

10 23. The method of claim 22, wherein said substrate surface comprises a gold coating and preparing for bonding comprises cleaning said gold coating.

24. The method of claim 23, wherein said anchoring segment is a thiol.

15 25. The method of claim 22, wherein said substrate surface comprises a metal coating selected from gold and aluminum, and preparing for bonding comprises cleaning and coating said metal coating with a functionalized aminothiol or aminosilane.

26. The method of claim 25, wherein said aminothiol or aminosilane is aminopropylsilane.

27. The method of claim 26, wherein said aminopropylsilane is functionalized with a maleimide.

20 28. The method of claim 27, wherein said substrate anchoring segment is a thiol.

29. The method of claim 25, wherein said anchoring segment is biotin.

30. The method of claim 25, wherein said aminothiol or aminosilane is functionalized with one of a hydrazide, aminoxy, N-hydroxysuccinimide, anhydride, aldehyde, disulfide, thiol, azide and phosphine.

31. The method of claim 25, wherein said metal substrate surface is further coated with an avidin protein beneath said anchoring segment.

32. The method of claim 31, wherein said avidin protein is selected from the group consisting of avidin, streptavidin, neutravidin and analogs.

5 33. The method of claim 31, wherein said anchoring group is biotin.

34. The method of claim 31, wherein said avidin protein is attached to the metal substrate surface via an NHS-LC-LC-biotin moiety.

35. The method of claim 21, wherein said peptidomimetic segment is a peptoid.

10 36. The method of claim 21, wherein said linker segment is selected from the group consisting of C2 – C100 aliphatic chains, polyethylene oxide, and orthogonal peptidomimetic or peptide oligomers.

37. A method of making an array comprising a plurality of different protein-binding agents stably associated with the surface of a solid support, said method comprising:

15 generating a library of protein-binding agents, comprising,

a substrate anchoring segment,

a peptidomimetic segment, and

a linker segment connecting and separating the anchoring and peptidomimetic segments;

20 distributing protein-binding agents from said library into individual storage receptacles for each different protein-binding agent;

preparing a plurality of said different protein-binding agents for binding to a solid substrate;

preparing a solid substrate having a substantially planar surface for binding with a plurality of said different protein-binding agents;

whereby said array is produced.

38. The method of claim 37, wherein said substrate surface comprises a metal coating selected from gold and aluminum, and preparing for bonding comprises cleaning and coating said metal coating with a functionalized aminothiols or aminosilane.

39. The method of claim 38, wherein said aminothiols or aminosilane is functionalized with a maleimide.

40. The method of claim 39, wherein said substrate anchoring segment is a thiol.

41. The method of claim 38, wherein said metal substrate surface is further coated with an avidin protein beneath said anchoring segment.

42. The method of claim 41, wherein said anchoring group is biotin.

43. A method of performing a differential binding assay, comprising:

labeling proteins in a protein-containing biological sample solution;

contacting an aliquot of said labeled protein-containing biological sample solution with an array according to claim 1;

analyzing the array to determine differential binding of proteins in the sample to protein-binding agents of the array.

44. The method of claim 43, wherein said peptidomimetic segment is a peptoid.

45. The method of claim 44, wherein each of said different protein-binding agents corresponds to a different source receptacle containing that agent.

46. The method of claim 45, further comprising selecting a protein-binding agent of interest based on the differential binding assay results.

47. The method of claim 46', further comprising sequencing the peptidomimetic segment of the selected protein-binding agent.

48. The method of claim 46', further comprising subjecting the peptidomimetic segment of the selected protein-binding agent to structural analysis by mass spectroscopy.

49. The method of claim 46, further comprising enriching the protein-containing biological sample solution with a protein which preferentially binds to the selected protein-binding agent by applying a second aliquot of the protein-containing biological sample solution to a separation column, said separation column comprising a chromatography support displaying the selected protein-binding agent.

50. The method of claim 49, further comprising sequencing said enriched protein.

51. The method of claim 49, further comprising subjecting the enriched protein to structural analysis by mass spectroscopy.

52. The method of claim 43', further comprising contacting an aliquot of said biological sample solution containing labeled cDNA or messenger RNA with a DNA array, analyzing the DNA array to determine differential binding of nucleic acids in the sample to elements of the DNA array, and comparing the differential binding results of the two arrays to identify correlations in gene activity and protein expression.

53. A kit for use in performing a differential binding assay according to claim 43', said kit including an array comprising:

a solid substrate having a substantially planar surface;

a plurality of different protein-binding agents bound to said substrate, each of said protein-binding agents comprising,

an anchoring segment stably bound to the substrate surface,

a peptidomimetic protein-binding segment, and

a linker segment connecting and separating the anchoring and peptidomimetic segments.

54. A mixed array of protein-binding agents stably attached to the surface of a solid support, said array comprising:

5 a solid substrate having a substantially planar surface;

a plurality of different protein-binding agents bound to said substrate, each of said protein-binding agents comprising,

an anchoring segment stably bound to the substrate surface,

a peptidomimetic protein-binding segment, and

10 a linker segment connecting and separating the anchoring and peptidomimetic segments; and

a plurality of different antibodies bound to said substrate.